PRIME-XV Dendritic Cell Maturation CDM

Catalog #	Product	Size
91146	PRIME-XV Dendritic Cell Maturation CDM	500 mL liquid Additional package sizes are available at request

Intended Use

For research or further manufacturing use only. Not for injection or diagnostic procedures.

Production Description

PRIME-XV Dendritic Cell Maturation CDM is a chemically-defined, animal component-free medium optimized for *in vitro* culture and differentiation of human monocyte-derived dendritic cells. This medium contains L-glutamine but does not contain antibiotics and phenol red, and can be further supplemented according to desired applications.

Quality Assurance

All quality control test results are reported on a lot specific Certificate of Analysis which is available at www.irvinesci.com or upon request.

Shipping

This product is shipped at 2-8°C. Upon receipt, store immediately at 2-8°C and protect from light.

Storage Instructions and Stability

Handle using aseptic techniques to avoid contamination. Unopened 500 mL PET bottle liquid medium is stable for 18 months from date of manufacture, as indicated on the label, when stored at 2-8°C and protected from light. Once opened, the product should be stored at 2-8°C in the dark and used within 4 weeks. Do not use after the assigned expiration date, not validated for use beyond the unopened expiry shelf life. Please refer to the Safety Data Sheet for information regarding hazards and safe handling practices.

Directions for Use

PROTOCOL FOR DENDRITIC CELL MATURATION

The following protocol is optimized for the differentiation and maturation of human dendritic cells from enriched $CD14^+$ monocytes using PRIME-XV Dendritic Cell Maturation CDM supplemented with the standard cytokine cocktail: GM-CSF, IL-4, TNF-α, IL-6, IL-1β, and PGE_2 (Jonuleit et al. Eur J Immunol 1997;27:3135-42.). Further optimization may be required depending on the cell type and application, such as culture duration and cytokine combination.

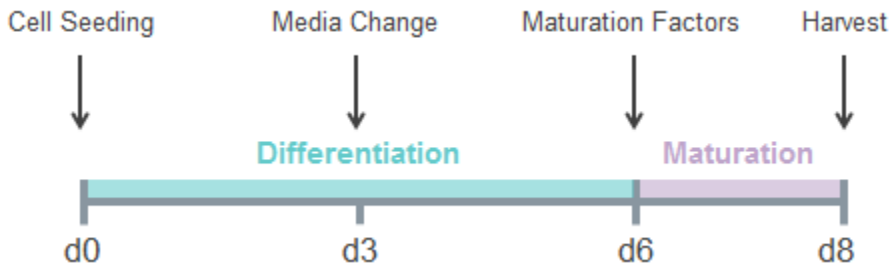
COMPLETE MEDIUM PREPARATION

A two-step approach with three feeding events over a period of 8 days is outlined here. The first two feedings on day 0 and 3 require the use of differentiation medium while the third feeding on day 6 requires the use of maturation medium, details of which are outlined below. *Note: make fresh respective media on the day of each feeding event.

1. To prepare **Differentiation Medium**, add appropriate amount of PRIME-XV Dendritic Cell Maturation CDM to a tube and supplement with GM-CSF (400 IU/mL) and IL-4 (1000 IU/mL). Mix thoroughly and warm up to 37°C prior to use.

2. To prepare **Maturation Medium**, add appropriate amount of PRIME-XV Dendritic Cell Maturation CDM to a tube and supplement with GM-CSF (400 IU/mL), IL-4 (1000 IU/mL), TNF-α (500 IU/mL), IL-6 (1000 IU/mL), IL-1β (1700 IU/mL), and PGE_2 (1 μg/mL). Mix thoroughly and warm up to 37°C prior to use.

3. **Note:** The concentrations listed are final concentrations of each respective cytokine after cell seeding. Preparation requirements may vary depending on media change setup according to end user application.



CELL PREPARATION AND PLATING

The following is a guideline based on the use of negatively enriched cryopreserved monocytes. Specific procedures provided by the respective cell supplier may also be utilized according to tissue source and user preference and need.

1. Pre-warm appropriate amount of PRIME-XV Dendritic Cell Maturation CDM in a 37°C water bath.

2. Add 9 mL of pre-warmed medium to a 15 mL conical tube.

3. Carefully remove the frozen vial of monocytes from liquid nitrogen and thaw in a 37°C water bath until the contents are almost completely thawed with a small amount of ice remaining.

4. Gently and slowly transfer the cell contents into the 15 mL conical vial containing pre-warmed medium.

5. Spin the cells down at 200x g for 5 min then resuspend the pellet in 5 mL of **Differentiation Medium** and perform a cell count.

6. Plate at a viable cell density of ~$4.7x10^5$ cells/cm^2 and bring up final volume of well/flask to the desired level with **Differentiation Medium.** Refer to the following table for respective cell densities and culture volumes according to desired culture vessel type:

Culture Vessel	Surface Area (cm^2)	Culture Volume	Seeding Density
96-well plate	0.32	200 µL/ well	$1.5x10^5$
48-well plate	0.95	300 µL/ well	$4.5x10^5$
24-well plate	1.9	500 µL/ well	$8.9x10^5$
12-well plate	3.8	1 mL/ well	$1.8x10^6$
6-well plate	9.5	2 mL/ well	$4.5x10^6$
T-25 flask	25	5 mL/ flask	$11.8x10^6$ cells
T-75 flask	75	15 mL/ flask	$35.3x10^6$ cells

7. Place the cells in a 37°C, 5% CO_2 humidified incubator and perform necessary media changes as outlined below.

MEDIA CHANGE AND MATURATION INDUCTION

The following is a general guideline which may be modified and adapted according to user preference and application.

1. On day 3 of culture, pre-warm appropriate amount of freshly prepared **Differentiation Medium** and perform a half medium change.

   Note: For larger cultureware such as flask setups, it may be necessary to collect and spin down the spent medium (200x g for 5 min) prior to resuspending the resulting cell pellet in appropriate amount of freshly prepared **Differentiation Medium** to ensure that any non-adherent cells that may be present are not discarded.

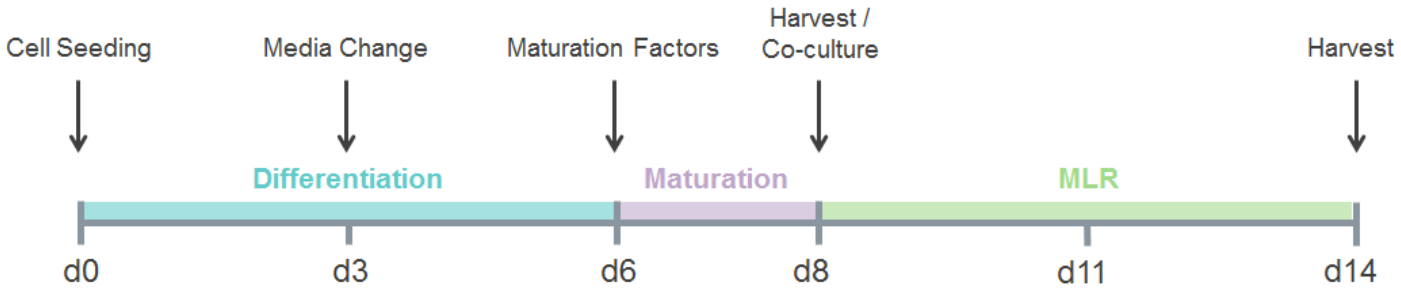
2. Incubate the cells in a 37°C, 5% CO_2 humidified incubator for 3 days to complete monocyte differentiation.
3. On day 6 of culture, pre-warm appropriate amount of freshly prepared **Maturation Medium** and perform a half medium change.

   Note: For larger cultureware such as flask setups, it may be necessary to collect and spin down the spent medium (200x g for 5 min) prior to resuspending the resulting cell pellet in appropriate amount of freshly prepared **Differentiation Medium** to ensure that any non-adherent cells that may be present are not discarded.

4. Incubate the cells in a 37°C, 5% CO_2 humidified incubator for 2 days to complete dendritic cell maturation.

PROTOCOL FOR MIXED LYMPHOCYTE REACTION (MLR)

The following is a guideline based on the approach utilized by Irvine Scientific during PRIME-XV Dendritic Cell Maturation CDM development. Day 8 mature Dendritic Cells were co-cultured with T cells as a measure of DC function. Modifications can be made according to user preference and application.



1. To prepare **MLR Medium**, add appropriate amount of PRIME-XV T Cell CDM to a tube and supplement with 50 U/mL IL-2 (2727 IU/mL). Mix thoroughly and warm up to 37°C prior to use.
2. Pre-warm appropriate amount of PRIME-XV T Cell CDM in a 37°C water bath.
3. Add 9 mL of pre-warmed medium to a 15 mL conical tube.
4. Carefully remove frozen vial of T cells from liquid nitrogen and thaw in a 37°C water bath until the contents are almost completely thawed with a small amount of ice remaining.
5. Gently and slowly transfer the cell contents into the 15 mL conical vial containing pre-warmed medium.

6. Spin the cells down at 200x g for 5 min then resuspend the pellet in freshly prepared **MLR Medium** and perform a cell count.

   Note: Adjust volume of cell suspension to dispense desired number of cells per 100 μL volume based on target T:DC ratio.

7. Dispense 100 μL of cell volume accordingly to a 96-well round bottom plate and place in 37°C, 5% CO_2 humidified incubator until dendritic cells are ready for plating.

8. Dissociate matured dendritic cells (Day 8) from plate and perform a cell count.

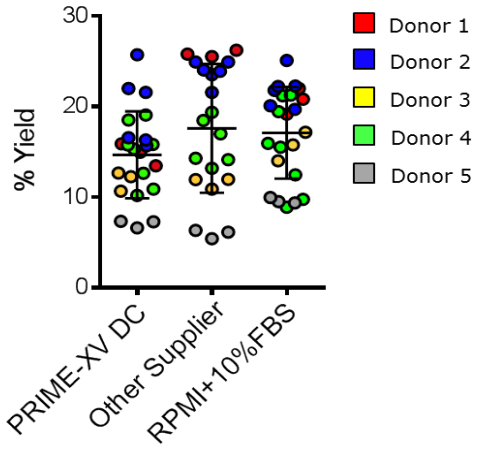
   Note: Depending on desired application, enzymatic dissociation can be utilized to ensure dissociation of both adherent and non-adherent cells from well.

9. Spin the cells down at 200x g for 5 min then resuspend the pellet in appropriate amount of freshly prepared **MLR Medium** and transfer to respective wells of 96-well round bottom plate containing T cells plated according to above instructions.

   Note: Desired volume is 100 μL of cell suspension per well. Cell density will vary based on desired T:DC ratio

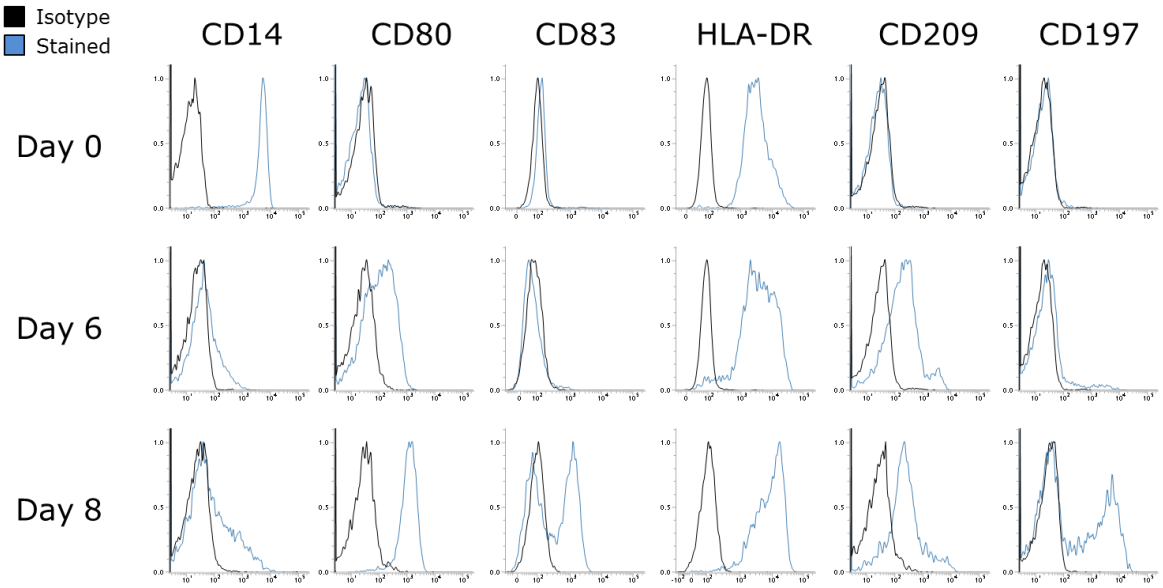
10. Incubate the cells in a 37°C, 5% CO_2 humidified incubator for 5-6 days until ready for harvest. Perform a half media change on day 2 or 3 of culture using freshly prepared **MLR Medium**.

Sample Data



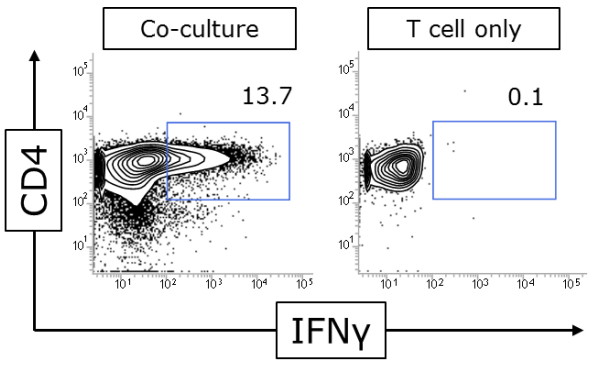
PRIME-XV Dendritic Cell Maturation CDM shows comparable yield of monocyte-derived dendritic cells.

Negatively enriched $CD14^+$ monocytes were cultured for 6 days in the presence of GM-CSF and IL-4 to induce differentiation and subsequently cultured for 2 more days in TNF-α, IL-1β, IL-6, and PGE_2 to induce maturation for a combined culture duration of 8 days in each respective media. The resulting cells were harvested and analyzed by flow cytometry for viable cell density (propidium iodide exclusion and count beads) and CD14 expression. Comparable monocyte-derived dendritic cell (Mo-DC) yield utilizing up to 5 donors was observed with respects to other supplier media and RPMI+10% FBS control. Yield is represented as total viable cell yield of $CD14^-$ cells on day 8 with respects to the starting number of $CD14^+$ monocytes plated on day 0.



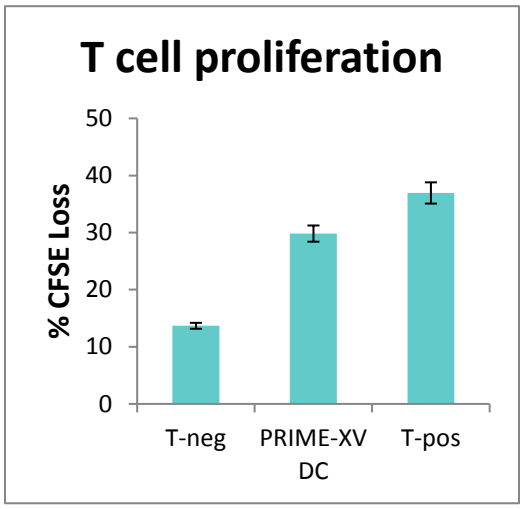
PRIME-XV Dendritic Cell Maturation CDM surface marker expression profile.

Negatively enriched $CD14^+$ monocytes were cultured for 6 days in the presence of GM-CSF and IL-4 to induce differentiation and subsequently cultured for 2 more days in TNF-α, IL-1β, IL-6, and PGE_2 to induce maturation for a combined culture duration of 8 days in each respective media. The cells were harvested and analyzed by flow cytometry for surface marker expression at day 0, day 6, and day 8 of culture.



PRIME-XV Dendritic Cell Maturation CDM generates dendritic cells capable of inducing T cell polarization (MLR protocol).

Day 8 Mo-DCs cultured in PRIME-XV Dendritic Cell Maturation CDM were harvested and co-cultured with allogeneic $CD4^+$ T cells at a 1:10 (DC to T) ratio and cultured in a commercially available T cell medium (such as PRIME-XV T Cell CDM - 91154-1L) supplemented with IL-2 for 6 days prior to flow cytometry analysis.



PRIME-XV Dendritic Cell Maturation CDM generates dendritic cells capable of inducing T cell proliferation (MLR protocol).

Day 8 Mo-DCs cultured in PRIME-XV Dendritic Cell Maturation CDM were harvested and co-cultured with allogeneic $CD4^+$ T cells at a 1:20 (DC to T) ratio and cultured in a commercially available T cell medium (such as PRIME-XV T Cell CDM - 91154-1L) supplemented with IL-2 for 6 days prior to analysis. T cell stimulatory capacity was confirmed by loss of CFSE (n=3). $CD4^+$ T cells cultured without Mo-DC were cultured in IL-2 alone or supplemented with anti-CD28 to serve as negative and positive controls, respectively.

Related Products

Catalog #	Product	Size
91154	PRIME-XV T Cell CDM	1 L liquid
95112	Recombinant Human GM-CSF ACF	20 µg
95114	Recombinant Human IL-4 ACF	20 µg
95117	Recombinant Human TNF-α ACF	10 µg
95118	Recombinant Human IL-2 ACF	10 µg
95121	Recombinant Human IL-6 ACF	20 µg
91139	PRIME-XV FreezIS	10 mL, 100 mL

Technical Support

CONTACT US

For more information or assistance contact Customer Service at:

- Email: tmrequest@irvinesci.com
- Direct line: +1 800 577 6097

WEBSITE RESOURCES

Visit the website at www.irvinesci.com for technical resources and information including:

- Safety Data Sheets (SDS)
- Certificate of Analysis (CoA) (when available)
- FAQs
- Product literature
- Complete list of offices and contact information by country

FUJIFILM Irvine Scientific, Inc.
2511 Daimler Street, Santa Ana, California 92705 USA
Telephone: 1 949 261 7800 • 1 800 437 5706
Fax: 1 949 261 6522 • www.irvinesci.com